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## Scientific letter

**CD64, CD11a and CD18 leukocytes expression in children with SARS-CoV-2 multisystem inflammatory syndrome versus children with Kawasaki disease: Similar but not the same**

**Comparación de la expresión de CD64, CD11a y CD18 en leucocitos de niños con síndrome inflamatorio multisistémico relacionado con SARS-CoV-2 y enfermedad de Kawasaki: semejantes pero distintos**

Dear Editor,

The immune response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) appears to be a critical factor in the prognosis of coronavirus disease 2019 (COVID-19).<sup>1</sup> Generally, children are less affected and developed asymptomatic or mild forms. Despite this, pediatricians across Europe have described severe cases of the disease. Firstly recognized as "Kawasaki-like" processes and later named as pediatric multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS).<sup>2-4</sup> We have seen similar cases in our center adding to its clinical and analytical study the application of immunophenotyping by flow cytometry (FC).<sup>4</sup>

In this report, we describe CD64, CD18, and CD11a expression in three children with PIMS-TS and compare it with three cases of Kawasaki Disease (KD, years 2018 and 2019). The CD64 is a type I high-affinity receptor for the Fc fraction of the immunoglobulin G, located on the surface of monocytes, macrophages, dendritic cells, and neutrophils. Increased CD64 on the cell surface is related to the intensity of stimulation received by inflammatory cytokines. Additionally, CD18, also known as integrin β2, participates in leukocyte adhesion and signaling. CD11a associates with CD18 to form the lymphocyte function-associated antigen 1, or LFA-1. Expressed on leukocytes, this T cell integrin plays a central role in leukocyte cell-cell interactions and lymphocyte stimulation.

The cases' clinical trajectories are described in Table 1. The children were studied after informed consent obtained from their parents or legal guardians. The samples were collected in sterile EDTA at room temperature, refrigerated at 4°C and analyzed by FC within 24 h. Cell surface expression of CD64, CD18, and CD11a were measured by BD FACS Canto II flow cytometer (Becton Dickinson, New York, USA). CD64 (clone 10.1), CD18 (clone CBR LFA-1/2), and CD11a (clone HI111) monoclonal antibodies were obtained from Biolegend® (San Diego, CA, USA). Expressions were

measured in monocytes, neutrophils and lymphocytes were identified on a dot-plot and gated. Cell viability was confirmed by 7-AAD staining. At least 10,000 events were recorded for each sample. Flow-cytometry settings and samples were prepared according to manufacturer instructions. The intensity of CD64, CD18, and CD11a surface expression were measured as mean fluorescence intensity in arbitrary units (MFI).

The expression of CD64, CD11a, and CD18 are in Table 1. All PIMS-TS cases received methylprednisolone prior to FC, the KD cases were studied before receiving immunoglobulin. As main finding, we observe higher upregulation of the three proteins studied in SARS-CoV-2 patients. This response appears to be similar but higher than in KD.

Recent papers have described that a dysregulated immune response may result in inflammation and clinical worsening in COVID-19 cases. Our cases show high levels of CD64 expression. This expression is also higher than described in some autoinflammatory diseases, so it could indicate immune dysregulation. Cytokines such as IL-1b, IL-6, and TNF-α affect the presence of CD64 on the cell surface.<sup>5</sup>

Regarding the LFA-1, it is known that it plays a key role in leukocyte migration into tissues. Lymphopenia is a common finding in COVID-19 patients.<sup>1</sup> This situation may be linked to the migration of CD8+ lymphocytes to the infected tissues. As seen in Table 1, the CD11a upregulation in CD8+ is clear and could be linked to this process. Additionally, LFA-1 is involved in the process of cytotoxic T cell-mediated killing as well as antibody-mediated killing by granulocytes and monocytes. We observed an exacerbated CD8+ LFA-1 expression compared to KD cases.

The nonantimicrobial COVID-19 therapies proposed are intended to downregulate the immune system. The cytokine storm theory, coupled with analytical data that suggest immune dysregulation or macrophage activation syndrome justify these therapies. In our cases, all FC analyses were performed after almost four days following disease onset. Also, in one case FC was conducted in a patient with positive immunoglobulin G to SARS-CoV-2, which is not a precocious immune reaction.<sup>1</sup> The observation of high CD64 expression helped to detect a proinflammatory status and helped to take the decision of using immunoregulatory therapies.<sup>4</sup>

In summary, we compare for the first time the immunophenotype of children with severe SARS-CoV-2 infection versus children with KD. We observed significant but higher upregulation of CD64, CD18, and CD11a expression on leukocytes. Prospective studies with a higher number of cases should be conducted to confirm this observation.

**Table 1**

Clinical trajectories of Kawasaki disease and SARS-CoV2 infected children and cell expression of CD64, CD18, and CD11a.

Clinical trajectories of Kawasaki disease and SARS-CoV2 infected children									
Patient	Age in years	Sex	Days of fever	KD Clinical features at admission	KD Laboratory criteria	Echocardiogram	Treatment	Response	
KD1	3	Female	5	C, E, X, O	1, 2, 7, 8	Normal heart function	Ig 1d	No fever from beginning	
KD2	5	Female	5	C, E, X, O	1, 2, 6	Normal heart function	Ig 1d	Low grade fever less than 24 h	
KD3	3	Female	5	C, E, X, O	1, 2, 6, 8	Normal heart function	Ig 2d	Persistent fever after 48 h, requiring a second dose of Ig	
SARS-CoV2 1 (IgG antibodies to SARS-CoV-2)	9	Male	4	X	1, 6, 7	Normal heart function	Methylprednisolone (1 mg/kg/day)	Recovered, 10 days of PICU admission	
SARS-CoV2 2 (Confirmed by RT-PCR from nasopharyngeal swab)	11	Female	6	X, C	1, 6, 7	Depressed cardiac function	Methylprednisolone (1 mg/kg/day), Ig 1d, inotropic support	Recovered, 8 days of PICU admission	
SARS-CoV2 3 (Confirmed by RT-PCR from nasopharyngeal swab)	11	Male	9	X, C	1, 3, 6, 7	Normal heart function	Methylprednisolone (1 mg/kg/day), tocilizumab, inotropic support	Recovered, 4 days of PICU admission	
Cell surface expression as mean fluorescence intensity in arbitrary units of CD64, CD18, and CD11a.									
	% Neutrophils CD64+	Neutrophils CD64	Neutrophils CD11a	Neutrophils CD18	Monocytes CD64	Monocytes CD11a	Monocytes CD18	Lymphocytes CD8/CD11a	Lymphocytes CD8/CD18
KD1	78.1	3070	2354	5918	12049	6101	9136	2731	4821
KD2	78.7	2045	3323	16821	11222	12,721	17812	5313	6545
KD3	83.5	2315	3304	9529	9761	9411	15175	6608	11919
SARS-CoV2 1	99.6	14526	6123	3010	34065	18564	4762	21648	1703
SARS-CoV2 2	99.8	20940	9315	11679	51403	35081	13466	20414	1617
SARS-CoV2 3	99.6	17218	6458	6134	45095	19484	8695	33722	2124

KD: Kawasaki disease.

KD Clinical features: E: changes in limbs; X: polymorphous exanthema; C: bilateral conjunctival injection; O: changes in oral cavity or lips; L: unilateral cervical lymphadenopathy &gt;1.5 cm.

KD Laboratory criteria: 1 = CRP > 3 mg/dL, 2 = ESR > 40 mm/h, 3 = WBC count > 15,000/mm<sup>3</sup>; 4 = normocytic anemia for age; 5 = platelets after fever for 7 d > 450,000/mm<sup>3</sup>; 6 = ALT > 45 IU/L; 7 = albumin < 3.0 g/dL; and 8 = urine > 10 WBCs/high-power field.

Legend and experiment data: The children were studied after informed consent obtained from their parents or legal guardians. The samples obtained were collected in sterile EDTA at room temperature or refrigerated at 4°C and analyzed by FC within 24 h. Cell surface expression of CD64, CD18, and CD11a was measured by BD FACS Canto II flow cytometer (Becton Dickinson, New York, USA). CD64 (clone 10.1), CD18 (clone CBR LFA-1/2), and CD11a (clone HI111) monoclonal antibodies were obtained from Biolegend® (San Diego, CA, USA). Expressions were measured in monocytes, neutrophils, and lymphocytes. Cell viability was confirmed by 7-AAD staining. At least 10,000 events were recorded for each sample. Flow-cytometry settings and samples were prepared according to manufacturer instructions. Neutrophils, monocytes, and lymphocytes were identified on a dot-plot and gated. The intensity of CD64, CD18, and CD11a surface expression were measured as mean fluorescence intensity in arbitrary units (MFI).

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## Conflict of interest

No conflict interest.

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